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2.12. Sulforhodamine B (SRB) Assay

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An abbreviated version of this protocol was published in Evidence-based Complementary and Alternative Medicine : eCAM in Feb 2021

Extracts of Perilla frutescens var. Acuta (Odash.) Kudo Leaves Have Antitumor Effects on Breast Cancer Cells by Suppressing YAP Activity

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Detailed protocol

SRB staining

Materials

- **SRB solution (dye)**: 0.4% SRB (230162, Sigma-Aldrich) in 1% acetic acid diluted with auto ddH_2O
- **0.1% acetic acid (washing buffer)**: 0.1% acetic acid (695092, Sigma-Aldrich) in auto ddH_2O
- **10% TCA solution (fixation)**: 10% TCA solution (T0699, Sigma-Aldrich) in auto ddH_2O
- **10mM Tris base solution (elution)**: Trizma® base (T6066, Sigma-Aldrich) in auto ddH_2O

Procedure

1. Cell seeding in 96well plates (seed a different number of cells depending on their cell sizes)
Example) 1x10^3 cells / each well in 96 well plate
2. Drug treatment: serial dilution (total media has to be 200 ul in each well)
Example) 0.01 – 0.1 – 1 – 2 – 5 – 10 – 20 – 40 – 60 – 80 – 100 – 1000
3. Incubation in 37°C incubator 12 to 16 hours
4. Harvest each plate in once every two days
1) Suction media carefully
2) Put 100 ul of 10% TCA solution in each well by multichannel pipette
3) Incubate the plate in 4°C for 1 h (dark)
4) washing TCA solution by D.W about 3 to 5 times
5) Add 80ul of 0.4% SRB solution using by multichannel pipette
6) Incubate the plate in the dark for 30 min
7) Remove the SRB solution with a 0.1% acetic acid buffer (washing)
8) Dry for 1h
9) Add 150ul of Trizma base solution and shaking 1 h to resolve the staining
10) Measure the absorbance in 540 nm
11) Analysis the raw data

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Kim, C. , Jeong, H. and Mo, J. (2023). 2.12. Sulforhodamine B (SRB) Assay. Bio-protocol Preprint. bio-protocol.org/prep2205.
2. Kim, C., Shin, Y., Choi, S., Oh, S., Kim, K., Jeong, H. and Mo, J.(2021). Extracts of Perilla frutescens var. Acuta (Odash.) Kudo Leaves Have Antitumor Effects on Breast Cancer Cells by Suppressing YAP Activity. Evidence-based Complementary and Alternative Medicine : eCAM 0(0). DOI: 10.1155/2021/5619761